Published in final edited form as:

Math Biosci. 2017 May ; 287: 12–23. doi:10.1016/j.mbs.2016.10.002.

The dual role of autoimmune regulator in maintaining normal expression level of tissue-restricted autoantigen in the thymus: A modeling investigation

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Abstract

The expression level of tissue-restricted autoantigens (TSA) in the thymus is crucial for the negative selection of autoreactive T cells during central tolerance. The autoimmune regulator factor (AIRE) plays an important role in the positive regulation of these TSA in medullary thymic epithelial cells and, consequently, in the negative selection of high-avidity autoreactive T cells. Recent studies, however, revealed that thymic islet cell autoantigen (ICA69) expression level in non-obese diabetic (NOD) mice, prone to developing type 1 diabetes (T1D), is reduced due to an increase in the binding affinity of AIRE to the Ica1-promoter region, which regulates ICA69 protein synthesis. This seemed to suggest that AIRE acts as a transcriptional repressor of Ica1 gene in the thymus, causing down regulation in the expression level of ICA69. To investigate this hypothesis and the apparent dual role of AIRE in negative selection, we develop a series of mathematical models of increasing complexity describing the temporal dynamics of self-reactive T cells, AIRE-mRNA and AIRE-(in)dependent thymic TSA-associated genes. The goal is to understand how changing the binding affinity of AIRE to Ica1-promoter affects both T-cell tolerance and the dual role of the transcription factor. Using stability analysis and numerical computations, we show that the model possesses a bistable switch, consisting of healthy and autoimmune states, in the expression level of Ica1 gene with respect to AIRE binding affinity, and that it can capture the experimentally observed dual role of AIRE. We also show that the model must contain a positive feedback loop exerted by T cells on AIRE expression (e.g., via lymphotoxin released by T cells) to produce bistability. Our results suggest that the expression-level of AIRE-mRNA in the healthy state is lower than that of the autoimmune state, and that negative selection is very sensitive to parameter perturbations in T-cell avidity.

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Keywords

Thymic negative selection; tissue-restricted antigens; autoimmune regulator; mathematical model; bistable switch; T-cell avidity

1. Introduction

The thymus is one of the central lymphoid organs dedicated to T-cell maturation and development. It is where T-cell progenitors originating from the bone marrow acquire their receptors (TCRs) that are essential for T-cell function within the immune system, and differentiate into multitude of T-cell types. These thymocytes proliferate at immense rates, but only 2-4% of their total number leave this organ each day; the vast majority of them die by apoptosis in the thymic medulla via a process called negative selection [1]. This process is crucial for removing thymocytes whose TCRs possess high binding affinity to self-antigens, making tolerance to such organ specific molecules plausible by preventing autoreactive high-avidity T cells from escaping into the periphery and causing autoimmune diseases [2, 1].

Type 1 diabetes mellitus (T1D) is a chronic disease characterized by an autoimmune destruction of pancreatic β -cells by cytotoxic T lymphocytes, particularly autoreactive CD4⁺ and CD8⁺ T cells that appear to escape negative selection in the thymus [3, 4]. β -cell specific autoantigens, such as pro-insulin, insulin, glutamic acid decarboxylase (GAD) and islet cell autoantigen 69 kDa (ICA69), are highly expressed in thymic epithelial cells, presumably to delete maturing T cells whose TCRs exhibit high binding affinity to these molecules [5, 6, 7]. Recent studies of non-obese diabetic (NOD) mice, prone to developing this immunological disorder with 60-80% susceptibility rate [8], have shown that NOD mice have reduced expression level of these proteins in the thymus, when compared to other strains of mice that are T1D-resistant [5, 9, 10]. In fact, exogenously overexpressing some of these autoantigens in the thymus and spleen of NOD mice resulted in a significant delay of disease progression in these mice [11], suggesting that the failure of negative selection may be implicated in disease onset.

The transcription factor autoimmune regulator (AIRE), abundantly expressed in the medullary region of the thymus, plays a crucial role in controlling the transcription of several genes that produce peripheral tissue-specific proteins (also called tissue-restricted antigens: TSA) [12, 13, 14, 15]. Animal models that lack the AIRE protein have reduced levels of TSA and they develop a multi-organ autoimmune disorder that mimics the human disease known as the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) [16, 17]. Indeed, in T1D, it was found that the expression level of several β -cell specific autoantigens in the thymic medullary epithelial cells (mTECs), such as insulin, are AIRE-dependent (i.e., upregulated upon an increase in AIRE-expression) [18]. In other words, AIRE has a variety of roles in the development of a healthy immune system, lying at the basis of thymic negative selection, which is a crucial immunological step. There are, however, a number of TSA that are not dependent on AIRE for their thymic transcription [13, 19].

Recent experimental evidence revealed that the increased binding affinity of AIRE to the Ica1 promoter region, responsible for Ica1 transcription and ICA69 protein translation, in NOD mice compared to C57BL/6 mice leads to a decrease in the level of expression of ICA69 in the medullary region of the thymus [5]. It was hypothesized that mutations in the single nucleotide polymorphism (SNPs) within the NOD Ica1 core promoter may determine AIRE-mediated downregulation of ICA69 expression in mTECs [5]. In other words, translation of a peripheral autoantigen is hindered and a greater activity of AIRE induces diabetes. This result is consistent with previous studies showing that other genes (a total of 432 of them) are upregulated in AIRE-deficient mTECs [20], suggesting that AIRE has a dual role in the process of negative selection, simultaneously upregulating and downregulating the transcription of various TSA-associated genes. How does AIRE exert such dual role of being an activator and repressor is the main focus of this study. We hypothesize that T cells in the thymus can alter AIRE synthesis or activity through a positive feedback mechanism regulated by cytokines. Lymphotoxin, a cytokine released by T cells, may be a candidate for such positive feedback loop affecting AIRE expression and its downstream target genes [21, 22, 23, 18, 24], a finding that has been challenged by other studies [20]. Our goal here is to develop a molecularly-explicit mathematical model to capture the dual effect of AIRE and explain the importance of the positive feedback loop in the (up- versus down-) regulation of autoantigens presented in the thymus.

To understand negative selection, several mathematical models analyzing central [25, 26, 27, 28, 29] and peripheral tolerance [30] have been developed. These population-based models focused on the dynamics of different classes of T cells and how their interactions affect positive and negative selection. Here, we extend these studies by investigating the underlying genetic regulation of these processes. We construct a gene network model (developed from a series of mathematical models of increasing complexity), similar to those presented in [31, 32], to describe the temporal dynamics of AIRE, up and downregulated TSA-associated and AIRE-(in)dependent genes (particularly Ica1 and Ins2) and T cells in the thymus. Our goal is to explain the differences between NOD and C57BL/6 mice strains pertaining to negative selection, and demonstrate that the dual effect of AIRE could be due to a bistable switch [33], representing the two levels of expression of the Ica1 gene, which disappears when the binding affinity of AIRE to its promoter region is increased. Our results reveal that such bistable behavior is manifested only if a positive feedback loop (via cytokines such as lymphotoxin) exerted by T cells on AIRE synthesis must be present. We will also show that the downstream effect of bistability on T cell tolerance persists, leading to the formation of two steady states: a healthy state representing successful deletion of autoreactive high-avidity T cells and an autoimmune state representing T cells escaping into the periphery. This will be done analytically by identifying the key factors that control bistability in the model, and numerically using bifurcation methods and stochastic simulations to verify the analytical results. The model thus provides a quantitative understanding of the role played by AIRE in this process.

2. Mathematical Models

We start the model development with the transcriptional regulation exerted by the AIRE protein at the level of the thymic medulla (see Fig. 1). Evidence for positive and negative

effects of AIRE can be found in the literature [20], but the exact regulatory mechanism associated with both pathways constitutes an active area of research [34, 35, 36]. In the following paragraphs, we derive expressions that represent the positive and negative actions of AIRE. These expressions, approximated by Hill functions under some steady state and kinetic assumptions, will be included in the equations describing the rates of change of the two main categories of autoantigens expressed by mTECs.

2.1. Transcriptional probability due to AIRE

In order to describe the dynamics of *Ica1*, we need to determine the right formalism associated with the transcription of this gene. To do so, we will express this process as a “probability” function in terms of the various conformations of the *Ica1*-promoter in relation to AIRE binding, as suggested by [5]. By letting n_1 be the number of binding sites for AIRE on the promoter region and P_A be the expression level of free (unbound) AIRE in the thymus, we obtain the following conformations of the promoter: (i) a “free state” (D_0) in which no AIRE is bound (here, the RNA polymerase II can bind and initiate transcription); and (ii) “bound states” ($D_i = D_0 : iP_A, i \in \{1, \dots, n_1\}$) in which i -molecules of AIRE are bound, $i \in \{1, \dots, n_1\}$. Based on this, we conclude that the probability of transcription is given by

$$\mathcal{R} = \frac{\sum_{i=0}^{n_1} \alpha_i D_i}{\sum_{i=0}^{n_1} D_i},$$

where $0 \leq \alpha_{n_1} \leq \alpha_{n_1-1} \leq \dots \leq \alpha_2 \leq \alpha_1 \leq \alpha_0 = 1$, and

$$D_0 + P_A \xrightleftharpoons[k_{off}]{k_{on,0}} D_1; D_1 + P_A \xrightleftharpoons[k_{off}]{k_{on,1}} D_2; \dots; D_{i-1} + P_A \xrightleftharpoons[k_{eff}]{k_{on,i-1}} D_i, \quad i \in \{1, \dots, n_1\},$$

where $k_{on,i}$ are the different binding rates for the AIRE proteins binding at the promoter level. Assuming that AIRE binding to the D_1 promoter conformation is a reaction occurring at a faster time scale than the rest of the thymic cellular processes, we can write the dissociation constants of P_A from the promoter, in all the aforementioned binding reactions, as

$$\begin{aligned} K_{D,0} &= \frac{k_{off}}{k_{on,0}} = \frac{P_A D_0}{D_1} \Rightarrow D_1 = \frac{P_A}{K_{D,0}} D_0 \\ K_{D,1} &= \frac{k_{off}}{k_{on,1}} = \frac{P_A D_1}{D_2} \Rightarrow D_2 = \frac{P_A D_1}{K_{D,1}} = \frac{P_A^2}{K_{D,0} K_{D,1}} D_0 \\ &\dots \\ K_{D,i} &= \frac{k_{off}}{k_{on,i}} = \frac{P_A D_i}{D_{i+1}} \Rightarrow D_{i+1} = \frac{P_A D_i}{K_{D,i}} = \frac{P_A^i}{\prod_{j=0}^i K_{D,j}} D_0, \quad i \in \{1, \dots, n_1 - 1\}. \end{aligned}$$

Thus, $\mathcal{R}$ can be written as

$$\mathcal{R} = \frac{\alpha_0 D_0 + \alpha_1 \left(P_A / K_{D,0} \right) D_0 + \cdots + \alpha_{n_1} \left(P_A^{n_1} / \prod_{j=0}^{n_1-1} K_{D,j} \right) D_0}{D_0 + \left(P_A / K_{D,0} \right) D_0 + \cdots + \left(P_A^{n_1} / \prod_{j=0}^{n_1-1} K_{D,j} \right) D_0} = \frac{\sum_{i=0}^{n_1} \alpha_i \left(P_A^i / \prod_{j=0}^{i-1} K_{D,j} \right)}{\sum_{i=0}^{n_1} \left(P_A^i / \prod_{j=0}^{i-1} K_{D,j} \right)}.$$

We will assume strong cooperativity for the AIRE binding sites at the promoter region (i.e., the promoter is more likely to be found only in the free and fully occupied states). In the case of the Ica1 promoter, AIRE acts as a repressor. Considering that transcription occurs only when the promoter is in the free state, we obtain the following expression for the probability function

$$\mathcal{R} = \frac{K_D^{n_1}}{K_D^{n_1} + P_A^{n_1}} := f(P_A), \quad \text{where} \quad K_D = \sqrt[n_1]{\prod_{j=0}^{n_1-1} K_{D,j}}. \quad (1)$$

Given that AIRE is also a transcriptional activator of other genes (upregulating the expression level of several β -cell specific autoantigens), we can express this particular process using a transcription probability function $\mathcal{J}$ given by

$$\mathcal{J} = \frac{P_A^{n_2}}{K_{\mathcal{U}}^{n_2} + P_A^{n_2}} := g(P_A), \quad \text{where} \quad K_{\mathcal{U}} = \sqrt[n_2]{\prod_{j=0}^{n_2-1} K_{\mathcal{U},j}}, \quad (2)$$

and $K_{\mathcal{U},i}$ are the relative binding affinities of each P_A to the promoter of AIRE-upregulated genes, for the n_2 binding sites on this promoter.

Note that coefficients n_1 and n_2 will take values larger than 1, in agreement with the cooperativity assumptions for the transcription factors P_A , binding at the available sites at the promoter region.

2.2. Ica-AIRE mRNA model

We begin first by considering a simple two-dimensional (2D) model describing the temporal dynamics of Ica1- and AIRE-mRNA expression levels, denoted by I and A , respectively. The equation for the Ica1 in this model consists of an AIRE-dependent transcription term with a rate σ_I and an AIRE-independent degradation term with a rate γ_I , as described by the equation

$$\frac{dI}{dt} = \sigma_I f(P_A) - \gamma_I I,$$

where $f(P_A)$ is defined in Eq. (2).

At the protein level, we assume that the expression levels of ICA69 (denoted by P_I) and AIRE (P_A) are directly proportional to their corresponding mRNA levels, an approximation that we will use throughout the paper. In other words,

$$P_I = \pi_I I \quad \text{and} \quad P_A = \pi_A A. \quad (3)$$

It follows that the 2D model for I and A is given by

$$\frac{dI}{dt} = \sigma_I \frac{K_I^{n_I}}{K_I^{n_I} + A^{n_I}} - \gamma_I I \quad (4a)$$

$$\frac{dA}{dt} = \beta_A - \gamma_A A, \quad (4b)$$

where $K_I = K_D/\pi_A$ and AIRE-mRNA has a constant transcription term given by β_A , and a

degradation rate γ_A . The I -nullcline of this system is given by $I = \frac{\sigma_I}{\gamma_I} \frac{K_I^{n_I}}{K_I^{n_I} + A^{n_I}}$, which is a strictly decreasing Hill function of A , whereas the A -nullcline is a vertical line given by

$A = \frac{\beta_A}{\gamma_A}$. Thus, the two nullclines intersect at one steady state, given by

$$(I_*, A_*) = \left(\frac{\sigma_I}{\gamma_I} \frac{K_I^{n_I}}{K_I^{n_I} + (\beta_A/\gamma_A)^{n_I}}, \frac{\beta_A}{\gamma_A} \right).$$

Notice that the model can only produce one steady state regardless of the values given to the AIRE binding affinities ($1/K_I$) or the steepness of the I -nullcline determined by the number of AIRE binding sites on the promoter (i.e., the Hill coefficient n_I). In fact, changing K_I will only shift the equilibrium point (I_*, A_*) smoothly along the A -nullcline, generating a bifurcation diagram that mimics the shape of the I -nullcline. Since the binding affinity of AIRE to the *Ica1*-promoter is a key to distinguishing C57BL/6 mice (the healthy group) from NOD mice (the autoimmune group), it follows that the model cannot produce a large discrepancy between the two strains of mice. This suggests that the model is lacking one essential feature; although a steep Hill function may be able to capture such switching behaviour, a large Hill coefficient (>4) is a choice that is physiological unreasonable. Therefore, we hypothesize, that bistability is the underlying mechanism regulating this process, generating a clear distinction between the two strains. In the following paragraphs, we expand the model to account for other aspects of this system that can produce bistability.

2.3. Inclusion of T-cell dynamics

The model thus far has not included the dynamics of the thymocytes directly. As shown in the feed-forward subsystem (black block) of Fig. 2, the inclusion of a T-cell pool entails the addition of another equation to system (4) describing the dynamics of thymocytes, denoted by T . It consists of a source term, representing bone marrow (BM)-derived progenitor cells migrating into the thymus, and two deletion terms, representing negative selection of high-avidity T cells, i.e., T cells that have TCRs with high affinity to autoantigens expressed as

pMHCs on mTECs. More specifically, the following equation must be included in the model,

$$\frac{dT}{dt} = \sigma_T - \left(\alpha_1 \frac{I^{m_1}}{K_1^{m_1} + I^{m_1}} + \gamma_T \right) T, \quad (5)$$

where σ_T is the entry rate of BM-derived progenitor cells into the thymus, $\alpha_1 I^{m_1} T / (K_1^{m_1} + I^{m_1})$ is the deletion term of high-avidity T cells that are reactive to ICA69 (assumed to be directly proportional to Ica1, as suggested by Eq. (3)), and $\gamma_T T$ is the deletion term of other high-avidity autoreactive thymocytes (assumed to be proportional to the size of the T-cell pool, with a turnover rate γ_T). Notice that the deletion term of ICA69-reactive T cells is given by a Hill function with a maximum deletion rate α_1 , half-maximum deletion K_1 (representing a threshold for T-cell avidity) and a Hill-coefficient m_1 . The extended model that combines Eq. (5) with system (4) also has a single steady state, given by

$$(I_*, A_*, T_*) = \left(\frac{\sigma_I}{\gamma_I} \frac{K_I^{n_1}}{K_I^{n_1} + A_*^{n_1}}, \frac{\beta_A}{\gamma_A}, \frac{\sigma_T}{\gamma_T + \alpha_1 I_*^{m_1} / (K_1^{m_1} + I_*^{m_1})} \right).$$

This means that extending the model through the addition of a T-cell pool, as defined by the feed-forward subsystem (black block) of Fig. 2, does not alter the dynamics of the model significantly and does not produce the bistability property that is essential for capturing the effects of AIRE on Ica1 dynamics. In fact, modifying Eq. (5) to

$$\frac{dT}{dt} = \sigma_T - \left(\alpha_1 \frac{I^{m_1}}{K_1^{m_1} + I^{m_1}} + \gamma_T \right) T - \epsilon T^2$$

by including the term $-\epsilon T^2$, which accounts for T-cell homeostasis due to competition and limited space, produces a unique steady state as well, given by

$$(I_*, A_*, T_*) = \left(\frac{\sigma_I}{\gamma_I} \frac{K_I^{n_1}}{K_I^{n_1} + A_*^{n_1}}, \frac{\beta_A}{\gamma_A}, \frac{\sqrt{\mathcal{L}_{A_*}^2 + 4\epsilon\sigma_T} - \mathcal{L}_{A_*}}{2\epsilon} \right), \text{ where } \mathcal{L}_{A_*} \text{ is a decreasing function}$$

of A_* , defined by $\mathcal{L}_{A_*} = \frac{\alpha_1 R^{n_1} K_I^{n_1 m_1}}{K_1^{m_1} (K_I^{n_1} + A_*^{n_1})^{m_1} + R^{m_1} K_I^{n_1 m_1}} + \gamma_T$.

It follows from the discussion above that the inclusion of the dynamics of T-cell pool alone (in the presence and absence of T-cell homeostasis) is not sufficient to produce bistability. In other words, we still need a (positive) feedback loop within the system to produce such behavior and account for the dual effect of AIRE on TSA.

3. Positive Feedback and Bistability

3.1. Nonscaled extended model

Experimental evidence suggests that thymocytes can alter AIRE synthesis or activity through a positive feedback mechanism regulated by cytokines, such as lymphotoxin, released by T cells. As shown in Fig. 2, the model will contain from now on, in addition to the feed-forward subsystem (black blocks) associated with Ica1-AIRE dynamics, a positive feedback loop exerted by T cells on AIRE (via lymphotoxin), as well as a new pathway associated with AIRE-(in)dependent TSA-mRNA (both of which are labeled with orange blocks). Assuming that lymphotoxin concentration is proportional to the number of T cells (T), we may conclude that the expression level of AIRE-mRNA (A) is governed by

$$\frac{dA}{dt} = \sigma_A \frac{T^{n_3}}{K_3^{n_3} + T^{n_3}} + \beta_A - \gamma_A A,$$

where β_A is the basal level of transcription of AIRE mRNA, and $\sigma_A T^{n_3} / (K_3^{n_3} + T^{n_3})$ is T-cell induced AIRE-mRNA transcription given by a Hill function with a maximum transcription rate σ_A , half-maximum transcription K_3 and a Hill coefficient n_3 .

The expression level of other TSA-mRNA (denoted by G), on the other hand, is governed by

$$\frac{dG}{dt} = \sigma_G g(\pi_A A) + \beta_G - \gamma_G G,$$

where $g(\pi_A A)$ is the transcription term for AIRE-dependent TSA-associated genes as defined by Eq. (2) ($P_A = \pi_A A$ according to Eq. (3)), β_G is the basal level of transcription of TSA-associated genes including those that are AIRE-independent, and $\gamma_G G$ represents the turnover rate of these (AIRE-dependent and -independent) genes.

The dynamics of T cells, based on the above equations, is thus given by

$$\frac{dT}{dt} = \sigma_T - \left(\alpha_1 \frac{I^{m_1}}{K_1^{m_1} + I^{m_1}} + \alpha_2 \frac{G^{m_2}}{K_2^{m_2} + G^{m_2}} + \gamma_T \right) T,$$

where σ_T and $\gamma_T T$ are as defined in Eq. (5), $\alpha_1 I^{m_1} T / (K_1^{m_1} + I^{m_1})$ is the deletion term of high-avidity T cells that are reactive to ICA69 (assumed to be proportional to Ica1, as suggested by Eq. (3)), and $\alpha_2 G^{m_2} T / (K_2^{m_2} + G^{m_2})$ is the deletion term of high-avidity T cells that are reactive to other AIRE-dependent and -independent TSA (also assumed to be proportional to the expression level of their associated genes).

We will combine the last three equations with the dynamics of Ica1 as shown in Eq.4(a), which will include a basal level of transcription of the Ica1 mRNA, denoted by β_I (similarly to the basal transcription rates β_G and β_A previously defined). Thus, we obtain the following nonscaled four-dimensional (4D) model

$$\begin{aligned}
\frac{dI}{dt} &= \sigma_I \frac{K_I^{n_1}}{K_I^{n_1} + A^{n_1}} + \beta_I - \gamma_I I \\
\frac{dG}{dt} &= \sigma_G \frac{A^{n_2}}{K_G^{n_2} + A^{n_2}} + \beta_G - \gamma_G G \\
\frac{dA}{dt} &= \sigma_A \frac{K_A^{n_3}}{K_A^{n_3} + T^{n_3}} + \beta_A - \gamma_A A \\
\frac{dT}{dt} &= \sigma_T - \left(\alpha_1 \frac{I^{m_1}}{K_1^{m_1} + I^{m_1}} + \alpha_2 \frac{G^{m_2}}{K_2^{m_2} + G^{m_2}} + \gamma_T \right) T, \quad (6)
\end{aligned}$$

where $K_G = K_{\mathcal{G}} / \pi_A$.

In the previous model, the variable G , defining the TSA-associated genes which AIRE-upregulated and AIRE-independent, is part of a negative feedback loop, since it leads to T cell deletion and less upregulation of AIRE via the cytokines. We also have that T activates A , which inhibits I , which inhibits T at its turn. Thus, these three components take part in the main regulatory positive feedback of the system.

3.2. Scaled model

Because many of the parameters of system (6) are unknown or difficult to estimate, we can nondimensionalize (rescale) the model and reduce the number of unknown parameters. This can be done by applying the following substitutions

$$\mathcal{I} = \frac{\gamma_I}{\beta_I} I, \quad \mathcal{G} = \frac{\gamma_G}{\beta_G} G, \quad \mathcal{T} = \frac{\gamma_T}{\sigma_T} T, \quad \mathcal{A} = \frac{\gamma_A}{\beta_A} A,$$

which are used to obtain the scaled model

$$\begin{aligned}
\frac{d\mathcal{I}}{dt} &= \gamma_I \left(1 + a \frac{\mathcal{K}_I^{n_1}}{\mathcal{K}_I^{n_1} + \mathcal{A}^{n_1}} - \mathcal{I} \right) \\
\frac{d\mathcal{G}}{dt} &= \gamma_G \left(1 + b \frac{\mathcal{A}^{n_2}}{\mathcal{K}_G^{n_2} + \mathcal{A}^{n_2}} - \mathcal{G} \right) \\
\frac{d\mathcal{A}}{dt} &= \gamma_A \left(1 + c \frac{\mathcal{T}^{n_3}}{\mathcal{K}_A^{n_3} + \mathcal{T}^{n_3}} - \mathcal{A} \right) \\
\frac{d\mathcal{T}}{dt} &= \gamma_T \left[1 - \left(\frac{\alpha_1}{\gamma_T} \frac{\mathcal{I}^{m_1}}{\mathcal{K}_1^{m_1} + \mathcal{I}^{m_1}} + \frac{\alpha_2}{\gamma_T} \frac{\mathcal{G}^{m_2}}{\mathcal{K}_2^{m_2} + \mathcal{G}^{m_2}} + 1 \right) \mathcal{T} \right], \quad (7)
\end{aligned}$$

where $a = \frac{\sigma_I}{\beta_I}$, $b = \frac{\sigma_G}{\beta_G}$, $c = \frac{\sigma_A}{\beta_A}$, $\mathcal{K}_I = \frac{\gamma_A}{\beta_A} K_I$, $\mathcal{K}_G = \frac{\gamma_A}{\beta_A} K_G$, $\mathcal{K}_A = \frac{\gamma_T}{\sigma_T} K_I$, $\mathcal{K}_1 = \frac{\gamma_I}{\beta_I} K_1$, and $\mathcal{K}_2 = \frac{\gamma_G}{\beta_G} K_2$.

We want to understand the possibly dynamics allowed by system (7). From a systematic point of view, $\mathcal{A}$ and $\mathcal{T}$ are the key players here, giving rise to a positive feedback loop (with $\mathcal{A}$ affecting the transcription of the autoantigens, which will affect $\mathcal{T}$ cells' escape, which will in turn affect $\mathcal{A}$ production). By setting $\mathcal{I}$ and $\mathcal{G}$ to steady state using quasi-steady state (QSS) approximation, we can reduce system (6) to a two-dimensional model involving $\mathcal{A}$ and $\mathcal{T}$ only, whose nullclines are given by

$$\mathcal{A}\text{-nullcline: } \mathcal{A} = 1 + c \frac{\mathcal{T}^{n_3}}{\mathcal{K}_A^{n_3} + \mathcal{T}^{n_3}}, \quad \mathcal{T}\text{-nullcline: } \mathcal{T} = \frac{1}{1 + f_I(\mathcal{A}) + f_G(\mathcal{A})},$$

where

$$f_I(\mathcal{A}) = \frac{\alpha_1/\gamma_T}{1 + \left(\frac{\mathcal{K}_1}{1 + \frac{a\mathcal{K}_I^{n_1}}{\mathcal{K}_I^{n_1} + \mathcal{A}^{n_1}}} \right)^{m_1}}, \quad \text{and} \quad f_G(\mathcal{A}) = \frac{\alpha_2/\gamma_T}{1 + \left(\frac{\mathcal{K}_2}{1 + \frac{b\mathcal{A}^{n_2}}{\mathcal{K}_G^{n_2} + \mathcal{A}^{n_2}}} \right)^{m_2}}.$$

Notice that $f_I(\mathcal{A})$ is a decreasing function of $\mathcal{A}$ and $f_G(\mathcal{A})$ is an increasing function of $\mathcal{A}$. This means that the slope of the $\mathcal{T}$ -nullcline can change sign and cause the $\mathcal{T}$ -nullcline to cross the $\mathcal{A}$ -nullcline at at most three distinct steady states, as shown in Fig. 3. Two of these steady states are stable (labeled $\mathbf{S}_1$ and $\mathbf{S}_3$ and highlighted by black rectangles) and one of them is a saddle (labeled $\mathbf{S}_2$ and highlighted by a gray rectangle). Note that trajectories representing the dynamics in the $\mathcal{A} - \mathcal{T}$ plane depict the basins of attraction of the two stable equilibria. This new model can produce bistability in a manner similar to what we would expect experimentally.

4. Numerical Simulations of the Extended Scaled Model

4.1. Parameter estimation

In order to better understand the dynamics of the extended scaled model (7), it is essential to estimate the parameter values appearing in this system. In this section, we provide detailed explanations on how these parameters were estimated using a combination of previously published work and/or fitting methods. As a summary of our estimation results, we present the numerical values for the parameters at the end of this section, in Table 3.

4.1.1. Kinetic parameters associated with the T-cell pool—To estimate T-cell dynamics, we use the experimental and modelling data from [27, 37, 38]. Note that, in the pool of T cells undergoing thymic negative selection, there are two subpopulations of T cells: namely, double positive (DP) and single positive (SP) thymocytes (either CD4^+ or CD8^+). Therefore, we will need to consider the dynamics of both subgroups (see Table 1) when estimating the rates for our model variable T .

- Entry rate in the thymus (σ_T)

We will use two estimates for σ_T : the first one is defined as the rate of thymic positive selection ($\sigma_{T,1}$), whereas the second one is the entry rate in the thymic medulla ($\sigma_{T,2}$).

1. The positive selection rate was experimentally determined to be $\sigma_{T,1} \approx 1.23 \times 10^5$ cells/hour $\times 24 = 2.95 \times 10^6$ cells/day [38].
2. The entry rates into all “cellular compartments” of the thymus, for the different T cells subpopulations, were estimated in [27]. For

our purpose, the T-cell entry rate into the thymic medulla is given by $\sigma_{T,2} \approx 1.4 \times 10^6$ cells/day.

$\sigma_{T,2}$ is a smaller estimate than $\sigma_{T,1}$, possibly because it does not account for other thymic processes, such as T-cell division, which occurs at a rate of 1-2 /day. Since extensive T-cell dynamic modeling is beyond the scope of this paper, we assume that the initial estimate, $\sigma_{T,1}$, is closer to the actual value of σ_T than $\sigma_{T,2}$.

- Maximum number of T cells that escape negative selection ($T_{\max}$)

In [27], the number of DP and SP T cells was estimated to be 94% of the total number of T cells in the thymus. It is also estimated that there are six times more cells undergoing negative selection than positive selection [38]. Thus, the maximum number of DP and SP T cells which can undergo negative selection (i.e., maximum number of T cells that can escape) is

$$T_{\max} = T_{DP+SP} = 94\% \times \left(3.1 \times 10^7 \text{ cells}\right) \times \frac{6}{(6+1)} = 2.5 \times 10^7 \text{ cells}.$$

It has been also suggested, in [37], that only 5% of the immature T cells generated in the thymus develop to form part of the peripheral T-cell repertoire, i.e., 95% of T cells are deleted. It follows that, in the nonscaled model, we have $T_{WT} = 5\% \times T_{DP+SP} = 1.25 \times 10^6$ cell, which is associated with the steady state of the scaled model by the equation

$$\mathcal{T}_{WT} = \frac{\gamma_T}{\sigma_T} T_{WT} = 0.4237 \gamma_T.$$

- T-cell deletion rates (α_1 , α_2 , γ_T)

- Total deletion rate due to negative selection ($\alpha_1 + \alpha_2 + \gamma_T$)

From [38], we know that the rate of negative selection is given by 6.99×10^5 cells/hour = 1.6776×10^7 cells/day. Since the deletion rates in the model have been kept in units of [/day], we need to consider the total deletion rate as

$$\alpha_1 + \alpha_2 + \gamma_T = \frac{1.6776 \times 10^7 \text{ cells/day}}{T_{\max}} = \frac{1.6776 \times 10^7 \text{ cells/day}}{2.5 \times 10^7 \text{ cells}} = 0.671/\text{day}, \text{ which is consistent with the model estimate of } 0.7/\text{day} \text{ obtained in [27]. From now on, the estimate } 0.671/\text{day} \text{ will be used as the total deletion rate.}$$

- Total deletion rate due to AIRE-independent processes (γ_T)

As shown in Table 1, it was determined in [27] that ~97% of DP cells are deleted via thymic selection. It means that

$$\alpha_1 + \alpha_2 \approx 0.97 \times 0.671/\text{day},$$

$$\gamma_T = 0.03 \times 0.671/\text{day} \Rightarrow$$

$\Rightarrow \alpha_1 + \alpha_2 \approx 0.651/\text{day}$ and $\gamma_T \approx 0.020/\text{day}$. Since AIRE plays a dominant role in TSA synthesis, we expect the upper bound for γ_T obtained above to be quite large and far from the physiologically-reasonable value.

– Deletion rates due to AIRE-dependent autoantigens (α_1, α_2)

To estimate these specific rates, we refer to the data provided by [20]. In the study, it was found, via microarray analysis, that there were 67 AIRE upregulated TSA genes, compared to 21 genes which appeared to be downregulated by overexpression of AIRE. Based on this, we have $\frac{\alpha_1}{\alpha_1 + \alpha_2} = \frac{21}{88} \approx 0.24$, or that 24% of the deletion rate is due to downregulated genes, vs. 76% due to the upregulated ones. We can write the previous expression as.

$$\begin{aligned}\alpha_2 &= \frac{21}{88}(\alpha_1 + \alpha_2) = \frac{21}{88}(0.671/\text{day} - \gamma_T) \quad \text{and} \\ \alpha_1 &= \frac{67}{88}(\alpha_1 + \alpha_2) = \frac{67}{88}(0.671/\text{day} - \gamma_T).\end{aligned}$$

In conclusion, if $\gamma_T \in [0, 0.020]$, then $\alpha_1 \in [0.155, 0.160]$ and $\alpha_2 \in [0.496, 0.511]$.

The remaining parameters related to T-cells dynamics (m_1, m_2, K_1, K_2) will be estimated based on the steady states of $\mathcal{I}$ and $\mathcal{G}$ and on the presence of bistability.

4.1.2. AIRE and AIRE-induced autoantigens dynamics—Here, we focus our analysis on estimating the parameters associated with AIRE ($\mathcal{A}$) and AIRE-induced autoantigens ($\mathcal{G}$), defined by the scaled model.

- Steady state values for AIRE and G ($\mathcal{A}_{WT}, \mathcal{G}_{WT}$)

In [39], the expression levels of AIRE and different AIRE-upregulated autoantigens of wild-type mice and $Tcra^{-/-}$ mutants were determined via PCR. These mutants, lacking both $CD4^+$ and $CD8^+$ T cells from the thymus, had a very small medulla. Based on the determined fold-increase in the expression levels of AIRE and various TSA (including SP1, SP2, Ins, MUP1 and Casein β) between wild-type (WT) and $Tcra^{-/-}$ mutant [39], we have, at

$$\text{steady state, } \frac{A_{WT}}{A_{Tcra-/-}} = 9 = \frac{\mathcal{A}_{WT}}{\mathcal{A}_{SS, \mathcal{I}=0}} = \frac{\mathcal{A}_{WT}}{1} \Rightarrow \mathcal{A}_{WT} = 9.$$

$$\frac{G_{WT}}{G_{Tcra-/-}} = 1.8|_{SP1} = 1.65|_{SP2} = 4.76|_{Ins} = 10.53|_{MUP1} = 12.5|_{Casein\beta} = [4.76, 6.28] = [median, mean].$$

Since the expression levels of various proteins in this model are assumed to be proportional to their corresponding mRNA level (see Eq. (3)), we can conclude from Eq. (7), that, at equilibrium,

$$\begin{aligned}\frac{\mathcal{G}_{WT}}{1 + \frac{b}{\mathcal{K}_G^{n_2+1}}} &\in [4.76, 6.28] \Rightarrow \\ \mathcal{G}_{WT} &= p \left(1 + \frac{b}{\mathcal{K}_G^{n_2+1}} \right), \quad \text{where } p \in [4.76, 6.28].\end{aligned}$$

In our numerical simulations, we use $p = 5$.

- AIRE-dependent transcription rate of $\mathcal{G}$ relative to its basal transcriptional level ($c/1$)

As shown in [18], after the administration of $1 \mu\text{g/mL}$ of agonistic LT β R for 6 hours, the expression level of AIRE and Ins2-mRNA in Ins2-expressing mTECs increased by 20- and 2.5-fold, respectively. According to our model, it follows that

$$\begin{aligned}
 & \frac{A_{WT+agonist}}{A_{WT}} \\
 &= 20 = \frac{\mathcal{A}_{max}}{\mathcal{A}_{WT}} = \frac{1+c}{9} \Rightarrow c \\
 &= 179 \quad \text{and} \quad \frac{G_{WT+agonist}}{G_{WT}} \\
 &= 2.5 = \frac{\mathcal{G}_{max}}{\mathcal{G}_{WT}} = \frac{1 + \frac{180^{n_2} b}{\mathcal{K}_G^{n_2} + 180^{n_2}}}{p \left(1 + \frac{b}{\mathcal{K}_G^{n_2} + 1} \right)}, \quad \text{which implies that } b \\
 &= \frac{(2.5p - 1)}{\left(\frac{180^{n_2}}{\mathcal{K}_G^{n_2} + 180^{n_2}} - \frac{2.5p}{\mathcal{K}_G^{n_2} + 1} \right)}. \tag{8}
 \end{aligned}$$

Since b is positive and $p > 4.76$ (as shown above), we have

$$\begin{aligned}
 & \frac{1}{\left(\frac{\mathcal{K}_G^{n_2}}{180} \right)^{n_2} + 1} > \frac{2.5p}{\mathcal{K}_G^{n_2} + 1} \Rightarrow \\
 & \mathcal{K}_G^{n_2} \left(1 - \frac{2.5p}{180^{n_2}} \right) > 2.5p - 1 \Rightarrow \\
 & \mathcal{K}_G > 180 \left(\frac{2.5p - 1}{180^{n_2} - 2.5p} \right)^{\frac{1}{n_2}}.
 \end{aligned}$$

By plotting the function

$$\mathcal{K}_G = 180 \left(\frac{2.5p - 1}{180^{n_2} - 2.5p} \right)^{\frac{1}{n_2}} \tag{9}$$

and recognizing that $p \in [4.76, 6.28]$, we obtain a range of values for $\mathcal{K}_G$ and b at each specific n_2 , as shown in Fig. 4(a) and (b), respectively. These ranges were used to provide parameter values for both $\mathcal{K}_G$ and b (see Table 2).

Parameter $\mathcal{K}_A$, representing the half-maximum transcription of AIRE, will be estimated using bistability.

4.1.3. Ica1 dynamics - bistability range—To estimate the parameters associated with the dynamics of Ica1 ($\mathcal{I}$), we rely mostly on the experimental data presented in [5]. Using RT-PCR analysis, it was shown that there is 5.5-fold change in the expression level of the protein ICA69 (P) when AIRE-deficient and NOD mice are compared, as well as a 2.7-fold

change in the expression level of the same protein between C57BL/6 and NOD mice. Thus, at steady state, we have

$$\frac{P_I (AIRE \approx 0)}{P_I (NOD mouse)} = \frac{5.5}{1} \quad \text{and} \quad \frac{P_I (C57BL/6)}{P_I (NOD mouse)} = \frac{2.7}{1},$$

which implies that

$$\frac{P_I (AIRE \approx 0)}{P_I (C57BL/6)} = \frac{5.5}{2.7} = \frac{\mathcal{J}_{\mathcal{A}=0}}{\mathcal{J}_{WT}} = \frac{1+a}{1 + \frac{a\mathcal{K}_I^{n_1}}{\mathcal{K}_I^{n_1} + 9^{n_1}}} =: f_1(a, \mathcal{K}_I, n_1), \quad (10)$$

where the protein expression level is assumed to be proportional to the mRNA expression level (see Eq. (3)). Notice that f_1 is a strictly decreasing function of $\mathcal{K}_I$, which means that by taking the limit as $\mathcal{K}_I \rightarrow 0^+$ (where $\mathcal{K}_I > 0$), we have, according to Eq.(10),

$$1+a > f_1(a, \mathcal{K}_I, n_1) = \frac{5.5}{2.7} \Rightarrow a > \frac{2.8}{2.7} \approx 1.037.$$

Furthermore, solving for a as a function of $x = (9/\mathcal{K}_I)^{n_1}$ in Eq.(10), we obtain

$$a(x) = \frac{1+x}{\frac{2.7}{2.8}x - 1}. \quad (11)$$

Since a is positive, we must have $x > 2.8/2.7$, or

$$0 < \mathcal{K}_I < 9 \left(\frac{2.7}{2.8} \right)^{1/n_1} < 9, \quad (12)$$

which represents an upper bound for $\mathcal{K}_I$.

It was also shown in [5] that the value of $\mathcal{K}_I$ for NOD mice is 7.25 smaller than that of C57BL/6 mice. Based on this, we conclude that, at the protein level, we have

$$\frac{P_I (C57BL/6)}{P_I (NOD mouse)} = \frac{2.7}{1} = \frac{\mathcal{J}_{WT}}{\mathcal{J}_{NOD}} = \left(1 + \frac{a}{1 + \left(\frac{9}{\mathcal{K}_I} \right)^{n_1}} \right) / \left(1 + \frac{a}{1 + \left(\frac{\mathcal{A}_{NOD}}{\mathcal{K}_I/7.25} \right)^{n_1}} \right).$$

Using $x = (9/\mathcal{K}_I)^{n_1}$ again, along with $y = \left(\frac{\mathcal{A}_{NOD}}{9/7.25} \right)^{n_1}$, we can solve for y and $\mathcal{A}_{NOD}$ as follows.

$$1 + \frac{a}{1+x} = 2.7 + \frac{2.7a}{1+xy} \Rightarrow \frac{2.7a}{1+xy} = \frac{a - 1.7(1+x)}{1+x} \Rightarrow y = \frac{1}{x} \left(\frac{2.7a(1+x)}{a - 1.7(1+x)} - 1 \right)$$

a is also a function of x , by Eq. 11, so

$$\mathcal{A}_{NOD} := f_2(x, n_1), \quad \text{where}$$

$$f_2(x, n_1) = \frac{9}{7.25} \left(\frac{1.7a + 2.7ax + 1.7(1+x)}{x(a - 1.7(1+x))} \right)^{\frac{1}{n_1}} = \frac{9}{7.25} \left(\frac{1+x}{x(1 - \frac{1.7}{2.8}x)} \right)^{\frac{1}{n_1}}$$

The denominator inside the n_1 th root cannot be negative. Therefore, we obtain an upper bound for x which is a lower bound for $\mathcal{K}_I$:

$$0 < x < \frac{2.8}{1.7} \Rightarrow \mathcal{K}_I > 9 \left(\frac{17}{28} \right)^{\frac{1}{n_1}}.$$

Using Eq. (12) and the expression $a(x)$ from Eq. (11), we can give now the bounds for x , $\mathcal{K}_I$ and a :

$$\frac{28}{27} < x < \frac{28}{17} \Rightarrow 9 \left(\frac{17}{28} \right)^{\frac{1}{n_1}} < \mathcal{K}_I < 9 \left(\frac{27}{28} \right)^{\frac{1}{n_1}}$$

and $a \in [4.5, \infty]$.

As an example, if $n_1 = 3$, $K_I \in [7.62, 8.89]$.

4.1.4. Transient dynamics—It has been established that in wild type mice, the expression level of AIRE and several TSA (such as Tff3, Ins2, Spt1 and Mup1) peak at day 11 after birth [40]. By digitizing the data associated with the fold changes in the expression level of AIRE and these TSA, we can use the genetic algorithm to fit the transient dynamics of the model to experimental data (see Fig. 6), where we assume as before that the expression level of these proteins is proportional to their mRNA level. Through this optimization technique, we can determine the initial conditions and the values of the parameters that are associated with the transient behavior of the scaled model given by system (7). These parameters are the time constants γ_F , γ_G and γ_A , and their estimated values are listed in Table 3.

4.1.5. Parameter estimation using bistability—It has been suggested in Section 3 that the bistability highlighted in Fig. 3 should be an important feature of the model. In fact, experimental evidence indicates that a 10-15% increase in thymic antigen levels sufficient to induce tolerance [6], which is a hint for bistability. By using this property of the model (i.e., by imposing the bistable structure of Fig. 3), we can provide estimates for the remaining parameters: m_1 , m_2 , $\mathcal{K}_A$, $\mathcal{K}_1$, $\mathcal{K}_2$ and γ_T . In other words, values for these parameters were

chosen in such a way that bistability of the model is attained. Numerical values of these parameters and all other parameters of the scaled model are listed in Table 3.

4.2. Bistability in the scaled extended model

In order to determine the full dynamics of the extended scaled model, we start by plotting the bifurcation diagram of its main variables with respect to its binding affinity of AIRE to the Ica1-promoter ($\mathcal{K}_I$). As shown in Fig. 7, a z-shaped bistable switch in the variables $\mathcal{A}$ (panel a) and $\mathcal{T}$ (panel b) is produced with respect to $\mathcal{K}_I$. The bistable switch consists of two branches of stable steady states (solid lines), and a branch of unstable saddle points (dotted lines), separating the two stable branches from each other and producing a hysteresis. The stable branches meet the unstable branches at saddle-node bifurcation points, encompassing a regime of bistability. The upper stable branch occurs at low $\mathcal{K}_I$ (i.e., when the binding affinity of AIRE to the Ica1-promoter is high) and corresponds to the autoimmune state, whereas the lower stable branch occurs at high $\mathcal{K}_I$ (i.e., at low binding affinity of AIRE to the Ica1-promoter) and corresponds to the healthy state. This scenario is equivalent to the one studied in [5], where the healthy state had a 7.25 fold difference in the binding affinity of AIRE in the Ica1 promoter for C57BL/6 mice, compared to results observed in NOD mice. As expected, the number of high-avidity autoreactive T cells escaping negative selection is high in the autoimmune state and low in the healthy state. Similarly, AIRE expression, represented by the scaled variable $\mathcal{A}$, is higher in the autoimmune state when compared to that of the healthy state. This suggests that an increase in the expression level of AIRE does not necessarily translate into the suppression of autoimmunity, and an optimal range of AIRE expression/activity must be maintained for a successful negative selection.

By doubling the value of $\mathcal{K}_G$, representing the half-maximum synthesis of $\mathcal{G}$, from 50 (black lines) to 100 (gray lines), we observe an upward shift in the upper branch of stable autoimmune states and no significant change in the lower branch of stable healthy states (see Fig. 7). The model shows that lowering the sensitivity of $\mathcal{G}$ with respect to $\mathcal{A}$ increases the pathogenic potential of the autoimmune state and increases the range of bistability seen previously (black lines). The presence of positive feedback, exerted by T cells on AIRE synthesis via lymphotoxin, plays a major role in generating these outcomes, through a highly nonlinear system, expressed by (7).

To further illustrate the effect of $\mathcal{K}_G$ perturbations in altering the dynamics of the model, we plot in Fig. 8 the two-parameter bifurcation of the scaled model with respect to $\mathcal{K}_I$ and $\mathcal{K}_G$ at different values of the Hill coefficients n_1 (a), n_2 (b) and n_3 (c). The two-parameter bifurcation, generated by varying two parameters and recording their effect on the two saddle-node points shown in Fig. 7, determines how the range of bistability is altered. From Fig. 8, we observe that increasing $\mathcal{K}_G$ past a certain value (close to 100), does not produce a significant change in the bistable range, for fixed values of n_i , $i = 1, 2, 3$. However, increasing the value of n_i , which reflects an increase in the steepness of the associated Hill-functions, enlarges the range of bistability by driving the curves of saddle-nodes further apart. Moreover, in the case of n_3 , we also observe (unexpectedly) a significant leftward shift in the bistable regime, indicating that the model is becoming more sensitive to AIRE. For example, in the case of Ica1, there are five polymorphisms that have been discovered at the

promoter level and differentiate the Ica1-promoter in the NOD vs C57BL/6 mice. Quantifying how many binding sites AIRE has on other TSA-related promoters can verify our modelling assumptions and predictions, allowing a better understanding of the sensitivity of TSA-related gene transcription to the AIRE factor.

We then extend this two-parameter bifurcation analysis to other set of parameters, including $\mathcal{K}_A$, which represents the half-maximum induction of AIRE transcription due to T cells, and $\mathcal{K}_1$ and $\mathcal{K}_2$, which are the half-maximum constants for deletion of T cells due to $\mathcal{I}$ and $\mathcal{G}$, respectively. When the parameters from each of the pairs given by $(\mathcal{K}_A, \mathcal{K}_1)$ and $(\mathcal{K}_2, \mathcal{K}_1)$, where $\mathcal{K}_i = \mathcal{K}_1 = \mathcal{K}_2$, are varied simultaneously, we obtain a few interesting results (see Fig. 9). First, the bistability regimes in both cases are enclosed within bounded regimes determined by the saddle-node curves, indicating that the two saddle-nodes of the bistable switch merge and the distinction between the healthy and autoimmune states is lost outside the bistable regime (close to the cusps of Fig. 9.a and b). This numerical observation predicts that bistability in the parameter regimes close to the cusps would be very difficult to detect experimentally. Second, the two-parameter bifurcation with respect to $\mathcal{K}_i$ (where $\mathcal{K}_1 = \mathcal{K}_2 = \mathcal{K}_i$) and $\mathcal{K}_1$ in Fig. 9.b shows significant sensitivity to perturbations in $\mathcal{K}_i$. More specifically, we see that the bistable regime is very narrow even at its widest portion, making small perturbations in $\mathcal{K}_i$ (e.g., diagonally across the widest portion of the bistable regime) capable of shifting the dynamics from the healthy state to the autoimmune state by decreasing the avidity of T cells to autoantigens (quantified by the magnitude of $\mathcal{K}_i^{-1}$, $i = 1, 2$). This result is consistent with experimental evidence linking low avidity with defective negative selection [4]. It highlights the role of avidity as a key factor in determining how T cells are selected for deletion.

4.3. Bimodality and stochastic dynamics

Bistability is frequently observed in molecularly-explicit networks and usually detected experimentally by observing bimodality in the expression level of the molecules of interest. The expression level of these molecules is typically measured using “fluorescence intensity” while modifying the dynamics of the network through targeting one of its components (e.g., through an agonist application). Here we show that bistability in the expression level of Ica1 and other TSA-specific genes (each represented by the scaled variables $\mathcal{I}$ and $\mathcal{G}$, respectively, and each thought of as a measure of fluorescence intensity) can also induce similar type of behavior; namely, the bistability property can be translated into a high fold change not only in the expression level of these genes, but also in the number of T cells in the thymus (represented by the scaled variable $\mathcal{T}$).

In order to capture this fold change, we simulate the stochastic time evolution of the scaled model, given in (7), using the Gillespie algorithm. Because the range of bistability seen in Figs. 7 and 8 is small, we target two parameter regimes that produce monostability, one of which corresponds to the autoimmune state (to the left of the bistable regime of Fig. 8) and the other corresponding to the healthy state (to the right of the bistable regime of Fig. 8). We use the parameter combination $\mathcal{K}_I = 0.01$ and $\mathcal{K}_G = 10^5$ for the former (i.e., the autoimmune regime), and the parameter combination $\mathcal{K}_I = 9$ (the upper limit of $\mathcal{K}$) and $\mathcal{K}_G = 0.01$ for the

latter (i.e., the healthy regime). We run our stochastic simulations 100 times for each case and use 10^5 simulation steps for each run. We then plot the average frequency of data points sampled by each run to compare the two regimes, as shown in Fig. 10. The first parameter combination ($\mathcal{K}_I=0.01$ and $\mathcal{K}_G=10^5$) produces histograms that peak at low levels of $\mathcal{I}$ and $\mathcal{G}$ (panel a). This causes $\mathcal{I}$ to exhibit two peaks, one of which is elevated, indicating a non-zero level of autoreactive high-avidity T cells and the failure of negative selection. For the other parameter combination ($\mathcal{K}_I=9$, $\mathcal{K}_G=0.01$) shown in panel b, we observe a very pronounced forward shift in the peaks of $\mathcal{I}$ and $\mathcal{G}$, corresponding to higher expression level of these genes (particularly $\mathcal{G}$), and a single peak in $\mathcal{I}$ at 0 which corresponds to no elevation in autoreactive high-avidity T cells. In other words, negative selection has been predominantly successful in the latter case.

These results can be tested experimentally by comparing the expression level of TSA, or TSA-specific genes, in mTECs of NOD and C57BL/6 mice and determining if there is a rightward shift in their expression level that is statistically significant when compared to each other. This will add evidence to the presence of positive feedback loop, as hypothesized in Fig. 2, which is a key element in generating the switching behavior assumed to be embedded in the data.

5. Discussion

Understanding autoimmune diseases at the genetic level remains a very difficult problem to undertake. For example, the involvement of many diabetogenic proteins in T1D, each regulated by a distinct set of processes, makes this disease particularly very complex and hard to analyze. Mathematical modeling can provide us with an alternative and promising platform to decipher some of these processes and examine the impact they have on disease onset and progression. In this paper, we developed an ordinary differential equation model to determine how the interaction of Ica1-promoter with the transcription factor AIRE affects autoimmunity in the context of T1D (a polygenic disease which is associated with multiple gene loci and haplotypes, the most important of which are the MHC genes). Environmental factors, such as infections that can trigger autoimmunity in predisposed individuals, were ignored in this modeling study.

Previous mathematical models in immunology focused on T-cell dynamics and provided interesting derivations about the positive and negative selection processes, notably that thymocytes death is almost negligible at the double negative stage, occurs mostly for double positive cells [29]. They have also discussed the experimental and mathematical evidences of various feedback mechanisms in the thymus, for example, how mature T cells affect death and differentiation other thymic components, through release of cytokines [27, 28]. Other papers highlighted the quantitative discrepancy in T-cells response in a healthy versus autoimmune states [41, 42, 38]. In this study, our goal is to bridge the simplified dynamics of T cells residing in the thymus, with the TSA-associated gene transcription, cytokine feedbacks and the presence of healthy-autoimmune states, due to defects in negative selection. Mainly, we employ bistability as a possible explanation of the presence of the two equilibria, in the context of the genetic branch and the autoantigen presentation events, regulated mainly by the transcription factor AIRE.

Islet cell autoantigen (ICA69) is a diabetogenic protein that is synthesized by Ica1 gene. Like insulin, it is expressed in the thymic epithelial cells with the intention of weeding out potential autoreactive T cells. The transcription factor AIRE is known to be a crucial component in the process of T-cell tolerance because it induces the expression of a large set of tissue-restricted antigens in medullary thymic epithelial cells for negative selection. The identification of a mutation in one single SNP within the NOD Ica1-promoter region, however, was found to increase the binding affinity of AIRE to Ica1-promoter and paradoxically repress its activity, leading to a reduction in the expression level of ICA69 in the thymus [5]. It was thought that this might be one of the causes of autoimmune T1D in NOD mice. Certainly conducting conditional Ica1 knock out experiments in the thymus may shed light into the mechanisms of negative selection and into the predisposition to autoimmunity. Here, we investigated this issue by examining AIRE binding affinity to Ica1-promoter in the context of a dynamic model. We showed analytically and numerically that the model can exhibit a bistable switch with hysteresis when a positive feedback loop exerted by T cells on AIRE-mRNA transcription (through, for example, lymphotoxin) is included in the model. The bistability was manifested by the existence of two stable steady states, an autoimmune state possessing an elevated expression level of AIRE-mRNA and a healthy state possessing reduced expression of AIRE-mRNA. This bistable behavior is consistent with and reflective of the presence of nondiabetic (healthy) and diabetic (autoimmune) mice, justifying the use of this hypothesis in the model. Certainly, experiments that would involve cell sorting and the study of various mutants with a defective presentation of autoantigens in the thymus could evaluate our parameter prediction and bimodality assumptions.

The model also revealed that increasing the binding affinity of AIRE to Ica1-promoter led to the disappearance of the healthy state through a saddle-node bifurcation point, leaving the autoimmune state as the only attractor of the model. Bistability was shown not to be significantly altered when the binding affinity of AIRE to the promoter region of other TSA-specific genes (represented by the variable $\mathcal{G}$) is increased. The two-parameter bifurcation method used to establish this result also showed that increasing the steepness of the Hill function describing the T-cell-dependent positive feedback loop of AIRE-mRNA transcription (through the Hill coefficient n_3), would enlarge the bistable region and shift this region to higher binding affinities of AIRE transcription factor. This surprising result is most likely due to the increased type of nonlinearity and the complexity of the positive and negative feedback loops included in this system.

The downstream effect of bistability on T-cell negative selection was investigated as well. We found that T cells also exhibit a bistable switch in their behavior in a manner similar to that seen in AIRE-mRNA, and displayed sensitivity to the half-maximum activation parameters $\mathcal{K}_1$ and $\mathcal{K}_2$. Using a two-parameter bifurcation, we showed that the bistable regime is very narrow and the model is very sensitive to perturbations in these parameters. Since these two parameters are indirect measures of T-cell avidity, we expect autoreactive T cells with higher avidity to be more susceptible to deletion, whereas those with lower avidity to trigger autoimmunity characterized by slow destruction of β -cells. This latter conclusion is in agreement with experimental observations [4].

One novel aspect of the model presented here is the inclusion of the feedback loop from T cells into the regulation of AIRE. Recent experimental evidence, suggests that the cytokine $\text{TNF-}\beta$, secreted by CD4^+ inflammatory T cells, also known as lymphotoxin, is involved in the regulation of AIRE expression [22, 43]. In fact, it has been shown that genetic variations in lymphotoxin affected AIRE levels and caused various autoimmune responses in mice strain subjects. On the other hand, when agonistic antibodies were used to stimulate the lymphotoxin receptors, AIRE expression was induced to normal levels and the self-antigens were re-expressed in the thymic medulla, impeding the development of autoimmune diseases. Therefore, lymphotoxin is another crucial element involved in the regulation of AIRE and, as a result, was taken into consideration when developing the model. The inclusion of this positive feedback from T cells not only caused a disparity in the affinity-dependent AIRE expression in the two steady states but also generated a range in the expression level of AIRE-mRNA within which autoimmunity was not invoked. Further analysis of all these details in combination with positive selection of thymocytes [44] in the context of autoimmunity, remains to be undertaken and would allow for a deeper understanding of the overall mechanism underlying thymic regulation of T-cell maturation and selection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by both the Natural Sciences and Engineering Council of Canada (NSERC) discovery grant to AK and the National Institutes of Health (Grants R01 DK53456, R01 DK56200 and R21 DK073724).

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Highlights

- Mathematical models are developed to study the effect of AIRE binding affinity on negative selection.
- We show that the models exhibit bistability with a healthy (successful negative selection) and autoimmune (failure of negative selection) states.
- We demonstrate that bistability is a reflection of the dual role of AIRE in the transcriptional regulation of tissue-restricted antigens.
- We predict that AIRE-mRNA expression level is lower in the healthy than in the autoimmune states.
- We conclude that negative selection is extremely sensitive to parameter perturbations in T-cell avidity.

Fig. 1.

Diagram depicting the negative selection of self-reactive T cells occurring in the thymic cortex, during embryonic-early life stages. Medullary thymic epithelial cells (*mTEC*) can express a great variety of autoantigens (red and orange circles) on their surface, via the MHC complexes (the triangles). These autoantigens are recognized by T-cell receptors (V-shapes) on the surface of T cells. We focus on β -cell specific autoantigens, encoded by mRNAs that are upregulated by AIRE (*blue*), for example, the *Ins2* gene, or downregulated by this transcription factor (*black*), like the *Ica1* gene.

Fig. 2.

A schematic diagram showing the proposed hypothesis for the effect of AIRE (A) as a transcription repressor of the *Ica1* gene (I) and as an activator for other tissue-restricted antigens (TSA, shown as G). The increased binding affinity of AIRE to the *Ica*-promoter leads to a decrease in ICA69 translation and a reduction in its expression level in mTECs, eliciting autoreactive T-cell maturation (T) and escape from the thymus. On the other hand, an increase in AIRE expression leads to an elevation of TSA in mTECs and an effective deletion of high-avidity autoreactive T cells. T cells release lymphotoxin that can upregulate the expression level and/or activity of AIRE. The black blocks represent the original feed-forward subsystem, whereas the orange blocks belong to the additional positive feedback loops through lymphotoxin as well as AIRE-dependent and -independent TSA. Dashed blocks represent model variables that are assumed to be at steady state, using QSS.

Fig. 3.

Diagram showing representative dynamics of the $\mathcal{A} - \mathcal{T}$ submodel, as determined by the $\mathcal{A}$ - (solid) and $\mathcal{T}$ - (dashed) nullclines. The intersection of these nullclines can occur at most three times, giving rise to three steady states labeled $\mathbf{S}_i$, $i = 1, 2, 3$. $\mathbf{S}_1$ and $\mathbf{S}_3$ are stable steady states (black rectangles) representing the healthy and autoimmune states, respectively, whereas $\mathbf{S}_2$ is an unstable saddle (gray rectangle) whose stable manifold acts as a separatrix between the basins of attraction of $\mathbf{S}_1$ and $\mathbf{S}_3$ (with associated trajectories shown as gray arrows). The numerical values for the parameters of system (7) are provided in Table 3, shown in the next section.

Fig. 4.

(a) The graphs of K_G versus p , as determined by Eq. (9). Dashed-dotted lines represent the physiological bounds estimated for p , using [39]. (b) The graphs of b versus K_G as determined by Eq. (8). Various integer values of n_2 have been used.

Fig. 5.

(a) The graph of a as determined by Eq. (11). Dashed-dotted lines represent the mathematical bounds estimated for x , using [39]. (b) The graphs of y versus x .

Fig. 6.

Fold change in the expression level of (a) $\mathcal{A}$, representing AIRE, and (b) $\mathcal{G}$, representing AIRE-induced TSA-associated genes, with peak at day ~ 11 . By digitizing the data in [40] (asterisks) and fitting model simulations (solid lines) to the data using the genetic algorithm, we can estimate the rate constants γ_I , γ_G , γ_A and the initial conditions of the four variables in the scaled model (7).

Fig. 7.

(a) One parameter bifurcation of $\mathcal{A}$ with respect to the binding affinity of AIRE to the Ica1-promoter ($\mathcal{K}_I$), depicting the differences in AIRE-mRNA expression between the two stable branches (black solid lines) representing the healthy steady states at high $\mathcal{K}_I$ and the autoimmune steady states at low $\mathcal{K}_I$. The two branches are separated by a branch of unstable saddle points (black dotted line), in the form of a hysteresis. The bistability range, where both the healthy and autoimmune states coexist, is smaller when AIRE binding affinity to the G-promoter is decreased by increasing $\mathcal{K}_G$ (gray solid and dotted lines). (b) A similar bifurcation diagram is seen for T cells when plotting the steady states of $\mathcal{T}$ with respect to $\mathcal{K}_I$.

Fig. 8.

The two-parameter bifurcation of the scaled model (7) with respect to $\mathcal{K}_G$ and $\mathcal{K}_I$, showing the bistable regime bounded by the saddle-nodes of Fig. 7, at different values of the Hill coefficients (a) n_1 , (b) n_2 (c) n_3 . Legends show the values used for n_1 , n_2 and n_3 . Notice that, in panel (c), there is a leftward shift in the bistable regime as n_3 is increased.

Fig. 9.

The two-parameter bifurcation of the scaled model (7) with respect to (a) $(\mathcal{K}_A, \mathcal{K}_I)$, and (b) $(\mathcal{K}_i, \mathcal{K}_I)$, $i = 1, 2$, where $\mathcal{K}_1 = \mathcal{K}_2 = \mathcal{K}_i$, showing the bistable regime bounded by saddle-nodes of Fig. 7. Sensitivity of the bistable regime with respect to changes in T-cell avidity $\mathcal{K}_i^{-1}$, $i = 1, 2$ is a key feature of the model.

Fig. 10.

Frequency of sampled data points for $\mathcal{I}$ (left panels), $\mathcal{G}$ (middle panels) and $\mathcal{T}$ (left panels) generated from averaging 100 stochastic simulations (each consisting of 10^5 simulation steps) of the scaled model obtained using the Gillespie algorithm. Two parameter combinations were used to observe the bimodality: (a) $\mathcal{K}_I=0.01$, $\mathcal{K}_G=10^5$, and (b) $\mathcal{K}_I=9$, $\mathcal{K}_G=0.01$. Notice the bimodality in $\mathcal{T}$ seen in panel (a), and the rightward shift in the frequency of $\mathcal{I}$ and $\mathcal{G}$ when going from panels (a) to (b).

Table 1

Parameters used for T-cell dynamics. The three columns represent the type of T cells participating in the process of negative selection. The top part of the table represents data reproduced from [27], whereas the bottom part depicts the results from [38]. The values in bold are used in the model.

	Double positive (DP)	CD4 ⁺	CD8 ⁺
Percentages	70-90%	7-15%	2-7%
(used in model)	82%	8%	4%
Entry rate (cells/day)	7 × 10⁵	4.5 × 10⁵	2.5 × 10⁵
Division rate $\left(\frac{\text{cell divisions}}{\text{cell} \times \text{day}}\right)$	1.0	0.02	0.01
(used in model)	1.0	0.02	0.02
Death Percentage	97%		
Death Rate (/day)	0.7	0.0	0.0
Positive selection rate (cells/day)	8.8 × 10 ⁶	2.35 × 10 ⁵	6.48 × 10 ⁵
Negative selection rate (cells/day)	1.2 × 10 ⁷	3.98 × 10 ⁶	7.92 × 10 ⁵

Table 2

$\mathcal{K}_G$ parameter as a function of n_2 coefficient.

n_2 value	1	2	3	4	5
$p = 4.76$	11.6716	3.3021	2.2172	1.8170	1.6124
$p = 6.28$	15.7406	3.8348	2.4497	1.9581	1.7118
Mean	13.7061	3.5684	2.3334	1.8875	1.6621

Table 3

Numerical values, definitions and units of parameters that correspond to the extended scaled model described by system (7).

Symbol	Definition	Value	Units	References
γ_T	Turnover rate of thymocytes	0.002	/day	fitted
α_1	Maximum T-cell deletion rate due to AIRE-downregulated TSA (ICA69)	0.1596	/day	[20, 27, 38]
α_2	Maximum T-cell deletion rate due to AIRE-upregulated	0.5094	/day	[20, 27, 38]
γ_1	Degradation rate of leal	0.158160	/day	[40]
γ_G	Degradation rate of TSA-associated genes (activated by AIRE)	0.313545	/day	[40]
γ_A	Degradation rate of AIRE mRNA	0.053824	/day	[40]
$\mathcal{K}_I$	Dissociation constant of P_A from $D_{1,I}$: P_A complex	7.8	mRNA copies volume	[5]
$\mathcal{K}_G$	Dissociation constant of P_A from $D_{1,G}$: P_A complex	100	mRNA copies volume	[18]
$\mathcal{K}_A$	Half-maximum induction of AIRE transcription due to T cells	0.031	mRNA copies volume	fitted
$\mathcal{K}_1$	Half-maximum deletion of effector T cells due to AIRE-downregulated TSA (ICA69)	2.1	mRNA copies volume	fitted
$\mathcal{K}_2$	Half-maximum deletion of effector T cells due to AIRE-upregulated TSA	2.1	mRNA copies volume	fitted
n_1	Hill coefficient of leal transcription	3	unitless	fitted
n_2	Hill coefficient of TSA-associated genes transcription	3	unitless	fitted
n_3	Hill coefficient of AIRE transcription	3	unitless	fitted
m_1	Hill coefficient of T-cell deletion due to AIRE-downregulated TSA (ICA69)	3	unitless	fitted
m_2	Hill coefficient of T-cell deletion due to AIRE-upregulated TSA	3	unitless	fitted
σ_T	T-cell entry rate into the epithelial region of the thymus	2.95×10^6	cells/day	[38]
a	$= \sigma_I \beta_I$ = Maximum rate of induction/Basal transcription rate for leal	5.2692	unitless	[5]
b	$= \sigma_G \beta_G$ = Maximum rate of induction/Basal transcription rate for TSA-associated genes	13.4721	unitless	[18]
c	$= \sigma_A \beta_A$ = Maximum rate of induction/Basal transcription rate for AIRE-mRNA	179	unitless	[18]